IDENTIFICATION OF DIFFERENT STRAINS OF CUCUMBER MOSAIC VIRUS BY SEROLOGICAL STUDIES

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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(Plant Virology)

BY

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Certificate

This is to certify that the dissertation entitled "Identifications of different strains of cucumber mosaic virus by serological studies", submitted by Miss Nuzhat Jabeen, is in partial fulfillment of requirements for the award of the degree of Master of Philosophy in Botany (Plant Virology) of the Aligarh Muslim University, Aligarh. The Research embodied in this dissertation is original and bonafide record of the work carried out under my supervision.

Qamar A. Naqvi

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(Nuzhat Jabeen)

"PLANTS SPEAK TO MEN BUT ONLY IN WHISPER; THEIR VOICE CAN BE HEARD BY THOSE WHO REMAIN CLOSE TO THEM"

DR. NORMAN E. BORLONG

Dedicated To Sweet Heart Abhi, Ammi Sisters and Brothers

Proclaim ! (or Read!)

In the name

Of thy Lord and Cherisher,

Who created

Created man, out of

A (mere) clot

Of congealed blood;

Proclaim! And thy Lord

is Most Bountiful.....

He Who taught

(The use of) the Pen

Taught man that

Which he knew not.

Sura (Alaq) XCVI 1-6

Al-Quran

CONTENTS

INTRODUCTION

The term "Vegetable" is usually applied to edible parts which store up food in the roots, stem, leaves and fruits. In fact they have no exact definition.

In India and all over the world, vegetables need no introduction as their recognition as an important article of daily human diet has come to be recognized fully. They play a very important role in the human diet, supplying some of the important things which are deficient in other food materials.

Vegetables needed as balanced diet have been observed through out the world. They are rich source of minerals like calcium, iron, vitamins especially vitamin-A, carbohydrates and proteins and also play an important role in neutralizing the acid substances, produced in the course of digestion of meat, cheese and other foods.

Vegetables are of value as roughage which promotes digestion and helps to prevent constipation. Besides they are also known to supply 25% of ascorbic acid and appreciable amount of thiamine, niacin and folic acid. Taking into consideration its value, they are thus used in correcting the

widespread mal nutrient in the under developed and developed countries.

Vegetables hold a high potential for combating the food shortage as their yield per unit area is more than five times of any cereal crop. In our country, the land under vegetable cultivation is many times more than in western countries but when compare the yield, we find ourselves trailing for behind than them. Of the various factors behind this low productivity, an important factor is the disease caused to them by various pathogens viz. fungi, bacteria, nematodes and viruses.

There are a large number of well defined groups of plant viruses. Members of each group generally differ in their natural and experimental host range, the symptoms they cause in the particular host species and their vector species. No doubt, the damage of every virus is unavoidable and huge but the loss caused by CMV is considerably very high and wide. CMV is known to infect all the important vegetables like cucumber, spinach, brinjal, tomato, chillies, radish, carrot, other crucifers and cucurbits. It has been isolated from many ornamentals and weeds: In India, more than 120 weeds have been reported as natural hosts for many viruses (Sastry 1984). *Amaranthus-viridis*, *Physalis minima*, *Solanum nigrum*, *Trianthema-monogyna*, *Boerhavia diffusa*, *Datura fastuosa*, *Celosia-*

argentea etc. are some of the reported hosts for CMV in India (Khurana, 1970); Joshi and Dubey, 1995. According to survey of its host range made by Komuro (1973) more than 191 species of 40 families have been found to be infected by CMV.

The CMV has a very wide host range. It is the most widely distributed plant virus. It is characterized by isometric particles measuring 28-30 nm is diameter (Shankar et al. 1971; Giri, 1985). The particle appears spherical with smooth outline and darkly stained central region due to the penetration of the negative stain.

The virus is efficiently transmitted in a non-persistent manner by several species of aphids. The most important among them are *Aphis craccivora, A.gossypii and Myzus persicae* (Bhargava and Bhargava, 1977; Dubey et al. 1974; Giri, 1985; Joseph and Ramanath 1978; Joshi, 1977; Rao, 1976; Ray chaudhuri and Verma 1975a; Singh et al. 1976; Tripathi and Joshi, 1985). The virus is known to be transmitted through seeds of several species of cucurbits, but in India, it is reported to be seed transmitted in only pumpkin and vegetable marrow (Mu khopadhyay and Saha, 1968; Sharma and Chohan, 1973).

The virus infects about 200 dicotyledonous and monocotyledons plants belonging to about 40 families (Francki

and Hatla, 1980). Some strains of virus in India possess a satellite RNA that attenuates system development (Giri, 1985). *Datura Stramonium* is a good diagnostic and *C. amaranticolor* a good assay plant. CMV causes mosaic disease in cucumber (Uppal, 1934; Bhargava and Bhargava, 1977), muskmelon. (Mayee et al. 1976; Sharma et al. 1984), Pumpkin (Reddy and Nariani 1963; Rao, 1976; Bhargava and Bhargava, 1977) and snake gourd (Dubey et al. 1974) in India.

The detailed information about its various properties like its host range, mode of transmission, biophysical properties, electron microscopy and serology is given below:

Description formula: --- $R/1: \frac{1.3}{1.8} + \frac{1.1}{18} + \frac{0.8+0.3}{18}$:S/S:/C,Ve/AP,

Discovery: Doolitte (1916) and Jagger (1916)

Geographical distribution: World wide, especially in temperate regions.

Virus group: Cucumovirus group.

Enveloped or non-enveloped: non enveloped.

Shape of particle: isometric

Size: 28-30 nm

Type of nucleic acid: Single stranded RNA. (SSRNA): Linear genome has four Parts: MW of largest: 2^{nd} : 3^{rd}: 4^{th}

$= 1.3\times10^6 : 1.1\times10^6 : 0.8\times10^6 : 0.35\times10^6$

Nucleic acid composition: G=24%; A=23%; C= 23%; U=30%

Composition of virion: Nucleic acid = 18%, proteins = 82%

Lipids and other compounds =0%

Dilution end point (DEP): 10^{-3} to 10^{-6}

Thermal inactivation point (TIP): 55^0C to 70^0C

Longevity in vitro: 1-10 days

Sedimentation coefficient: 1 compound, S_2O,

W=98.65 (-1.04×virus conc.)

Density (in caecium chloride): 1.367/cm^3

Isoelectric point: pH 5.5 (Q. Strain)

Replication: Independent, sub-genomic mRNA found in infected cells.

Serology: Poor immunogenecity, low salt in gel diffusion and non specific ppt in saline (best condition), ISEM.

Transmission: mechanical, seed (19 species)

Vectors (non-persistently by more than 60 species); Dodder (by atleast 10 species of cuscuta)

Host range: wide, 191 species in 40 families (Komuro, 1973)

Assay host: *Vigna unguiculata* (Crowley 1954), *Chenopodium amaranticolor* and *C.quinoa*

Diagnostic species: *Chenopodium amaranticolor, C. quinoa* (chlorotic and necrotic local lesions) *Cucumis sativus* (cucumber) systemic mosaic and stunting and *Nicotiana glutinosa*, *Vigna unguiculata*, *Lycopersicon esculentum* and *cucurbita moscheata* are suitable hosts.

Propagation species: *N.glutinosa* and *N.tabacum* cv. *Xanthi* (convenient for maintaining cultures), *N.tabacum* and *Cucumbita pepo* (suitable for virus source), but *N.clevelandii* (best for some strains)

Main disease: Mosaic of cucumber and cucurbits blight of spinach, fern leaf of tomato, mosaic of celery and mosaic of many other species of dicotyledonous crops, ornamentals and

weeds. Dwarfing and flower breaking is also reported (Smith, 1972).

Relationships: Tomato aspermy, peanut stunt, cowpea ringspot (Virus particles serologically related).

Remarks: The physical, antigenic and biological properties overlap the properties of some other viruses, inclusion bodies present are crystals and tonoplast particles found in cytoplasm.

Reviews: VIDE (Virus identification data exchange)

DPV-13, 201-215, 1979 (CMI/ASB Description of plant viruses, July 1979).

In the present study an attempt has been made to differentiate different strains of cucumber mosaic virus on the basis of biosphysical properties and host range. Detailed studies will be carried out for the identification of these strains in my Ph.D. work.

REVIEW OF LITERATURE

Almost all the vegetables have been reported to be infected by cucumber mosaic virus. Among most important are cucumber, tomato, spinach, Chilli, brinjal, potato and turnip.

Cucumber and other cucurbits

Doolittle (1921), Doolittle and walker (1925) and Walker (1925) reported a mosaic disease of cucumber from U.S.A. where they showed the role of weeds (Milk weed *Asclepias syric*

a) *Martyma Lousiana* and Pokeweed-*phytolacea decandra* and wild cucumber *micrampelis lobata*) and *capsicum annuum* and the insect vectors (aphid-*Myzus persicae* and beetle- *D. vittata*) in the spread of this disease. However, they later reported *Physalis hetero- phylla* and *P.subglabrata* are imp source of inoculum for cucumber mosaic virus.

Ainsworth (1934, 1935) reported mild mosaic or ordinary mosaic (named it as green mottle mosaic of cucumber), and yellow mosaic (named as yellow mottle mosaic) caused by cucumber virus 3 and cucumber virus 1, respectively. A third aucuba mosaic re-named yellow mosaic of cucumber was reported to be caused by cucumber virus 4, occurring naturally, only on cucumber. Cucumber virus 4 remained infective after a storage period of more than 9 months, tolerated a temperature

of 80^0C. The virus was mechanically transmissible to cumcumber and other cucurbits.

The occurrence of a mosaic disease of vegetable marrow and rock melon, (*cucumis melo var.cantalupensis*) was reported by Chamberlin (1939). He showed that the virus was transmitted mechanically as well as aphids, *Aphis gossypii*, *Myzus persicae* and *Macrosiphum solani*, retained its infectivity upto a thermal inactivation point of 60-66^0C, remained infective upto 4 days *in vitro* and had a dilution end point 10-3.

Rainio (1943) described same type of virus in Finland. The symptoms included protuberances on the aerial organs accompanied by arching and crinkling of the leaves and in severe infection, sterility. The aphids and centripedes were shown to be the vector of virus.

Occurrence of *cucumis* 2 (a strain of *cucumber green mottle mosaic virus*) on cucumber was reported by valentin (1958), from a nursery of Berlin (Germany). Affected plants showed stunted growth and occasional shrivelling of upper leaves vein clearing and pale yellow mosaic with asteroid spots, changing into silvery mosaic was observed on the affected leaves. The virus was sap transmissible persisted in dried material and remained infective in sap after a storage

period of 100 days. Thermal inactivation point ranged between 80-90^{0}C (10minute exposure).

Liem (1959) reported cucumis virus 2 (cucumber green mottle virus) on cucumber in the Netherlands.

A virus causing vein clearing, chlorosis and a green necrosis of cucumber in Jorden valley, Israel, was reported by Cohen and Nitzany (1960). They claimed it to be the first virus on cucurbits and the only plant virus known to be transmitted by white fly *Bemisia tabaci* as well as mechanically. Harpaz and cohen (1965) named this virus as cucumber vein yellowing virus (CVYM) to avoid confusion with viruses given to the name bottle gourd mosaic virus in India. Moskovets and Glushak (1968) reported cucumber mosaic virus as the causal agent of a virus disease of cucumber in Ukrane.

Protsenko (1969) gave a dercription of cucumber virus I (cucumber mosaic virus) and cucumber virus 2 (cucumber green mottle virus). The former usually appears one month after planting, over winters in the roots of perrinial weeds and is transmitted by aphids while the later is seed transmitted. The air temperature in glass house affected the symptoms of mosaic i.e. at over 25^{0}C, the mosaic observed was white and at lower temperature it was green.

Silberschmidt and Herbas (1969) observed rings on the leaves of *Allamanda calhartica* L. sap inoculation induced local lesions on *Chenopodium quinoa* and systemic symptoms on the leaves of *N.glutinosa* petunia hybrida and cucumber. The virus retained its infectivity upto 65^0C and concluded it to be a new strain of cucumber mosaic virus.

Demeski (1969) reported that cucumber mosaic virus is mechanically transmissible to summer squash in the glass house conditions.

Harvamek (1970) in a survey of cucumber diseases, found tobacco necrosis and five strains of cucumber mosaic virus as the only viruses attacking cucumbers in different localities of Czechoslovakia.

Verma et al. (1970) reported a mosaic disease of vegetable marrow similar to cucumis virus 1 (cucumber mosaic virus). Karl (1971) reported the *Dysaphis crataegi* a new non-persistent vector of cucumber mosaic virus.

Pelet and Hani (1971) described various methods of concentrating CMV, compared, and assayed 39 preparations. The methods gave heterogenous results except gel filtration on sepharose which gave highly infectious solutions showing a low level of contamination but a poor yield.

Twardowicz-Takusz Anna (1971) observed that the infection of cucumber in the pozanan area varied from 0.04% to 93%. The virus was detached from 10 to 27 vegetables, ornamentals and weeds examined from the vicinity of infected cucumber.

Peden et al. (1972) reported that the difference in the size of the soluble cucumber mosaic virus and tobacco ring spot virus induced RNA polymerases and in the solubilization of these enzymes from the particular fractions, may provide evidence for virus coded differences in the life cycles of the 2 viruses.

Boatman et al. (1973) compared the peanut stunt virus (PSV) with cucumber mosaic virus and concluded, peanut/stunt virus a strain of cucumber mosaic virus as the PSV had same properties as of CMV.

Devergne and Cardin (1973) used the technique like double diffusion agar to study the antigenic properties of 11 isolates of cucumber mosaic virus in France, Belgium, Italy and U.S.A. They found 12 catogories of antibodies with a fairly high specific corresponding to 12 possible epitops of the intact capsid of the virus, four serological type, ToRS and DTL were distinguished each with highly specific epitops and showed that

all isolates inducing rings pot lesions on xanthi tobacco belongs to the group To, R,S.

Sharma and Chohan (1973) reported the transmission of cucumis virus 1 and 3 through seeds of cucurbits. In seed transmission tests cucumis virus 1 (cucumber mosaic virus) was found to be seed borne in vegetable marrow, ash gourd (*Beninincasa hispida*) and pumpkin and cucumis virus 3 (cucumis green mottle virus) in bottle gourd (*Lagenaria siceraria*).

Sohara and Osaki (1974) purified cucumber mosaic virus by repeated precipitation with 8% PEG and 0.2M NaCl followed PEG solubilit gradient (0.06% PEG Stabilized in 35-50% sucrose). Centrifugation virus yield were 500-800 mg/kg fresh tissue. Purified preparations were highly infections and contained uniform virus particles. The sedimentation pattern showed a single peak, indicating that no aggregation occurred.

Chung et al. (1975) infected 145 plants (24, previously unreported) from 45 families with cucumber mosaic virus, but negative results were obtained with 23 spp. reported to be susceptible by previous workers.

Devergne and Cardin (1975) analysed the antibody composition of 37 antisera prepared against 16 virus isolates and found that 4 serological groups can be distinguished, CMV

TORS, CMV-DTL, TAL and PSV-V, each possessing specific antigenic patterns. The TAV (Tomato aspermyvius) group, which included all aspermy viruses is related to PSV-V and much more remotely to CMV (TORS).

Singh *et al.* (1977) reported the significant reduction in chlorophyll contents and increased respiratory loss in the leaves of cucumber infected by cucumber mosaic virus.

Bhargava & Bhargava (1977) reported pumpkin yellow-vein mosaic virus, pumpkin virus and cucumis virus 3 (cucumber green mottle virus) from 7 cultivated and 2 wild cucurbits from Gorakhpur (U.P.). They identified 3 str. of cucumber mosaic virus and 7 of water melon mosaic virus and concluded their close serological relationship.

Amemiya and Misawa (1977) studied the induction of resistance of cucumber by cucumber mosaic virus. They showed that virus multiplication in the cotyledons dishes of susceptible and resistant cultivars was similar during the initial stages of infection but the virus contents of later fell after 36h whereas that of the former remained constant. Treatment with actinomycin-D, α-amonitin or heat increased virus multiplication in the resistant cultivars.

Lout'eva Kosheleva and Konovalova (1978) reported the occurrence of cucumber mosaic virus in the field in Kuybyshev

region in Mid August. Losses were reported to be highest in the late crop protected against wind.

Lee et al. (1978) multiplied the cucumber mosaic virus (CMV) in the tobacco cv. KY-57 and purified it to give 24.25mg/ml.

The epidemiology survey of viruses affecting cucurbit crops in Massachusetls of Komm *et al.* (1978) showed that 17% infection was caused by cucumber mosaic virus.

Rahiman and Izadpanah (1978) identified the cucumber mosaic virus on the basis of transmission, host range, electron microscopy and serology Twelve isolates of cucumber mosaic virus from cucurbits Zinnia, petunia and viola hybrids were divided into four groups, according to the type of symptoms produced and into 2 serological categories.

Shanmyganathan (1980) in survey of the viral disease of three islands reported the occurrence of cucumber mosaic virus on cucumber and water melon virus on pumpkin.

Omar et al. (1982) isolated 3 strains of CMV, from naturally infected cucumber, beans and squash plants. Their studies revealed that CMV need atleast 48 hours to multiply in inoculated leaves, after which they could be detected in all leaves of the plant, showing the virus particles more with assimilates. No virus was detected in uninoculated leaves

24hours after inoculation. All components of cucumber and tomato fruits possessed the virus particles but at maturity the virus concentration decreased.

Kyriakopdou and Bem (1982) reported the occurrence of cucumber mosaic virus in squash from Greek. They isolated the virus and identified as CMV on the basis of host range, aphid, transmission, particle size and morphology, physical properties and serology. The virus was widely distributed throughout Greece on many cultivated and wild plants. The plants showed severe malformation and natural infection of *Globe artichoke* was noted.

Yamamoto and Ishii (1983) while studying aphid transmission in cucumber cultivars infected with water melon mosaic virus and cucumber mosaic virus reported that the virus in leaves inoculated with either virus showed a highly positive correlation with the rate of transmission of *Aphis gossypii*. It was noted that doubly infected leaves showed greater severity as compared to singly infected leaves with CMV. However, reverse was the case with WMV i.e. the leaves infected only with WMV showed higher severity as compared to the leaves infected with both the viruses. These results presented a clue to observe the difference between the concentration of the two viruses in leaves infected with both simultaneously.

Glass house trails of Erdiller and Ozyamar (1983) with cucumber seedlings grown in perlite and inoculated with cucumber mosaic virus (CMV) on cotyledons revealed that on Ist leaf the protein content was increased but respiration, starch and sugars was reduced compared with the healthy ones.

In an investigation, made by Dikova (1983) of cucumber cultivars to CMV, it was found that out of 89 culltivars inoculated with race B and C, 44 cultivars with race C of virus in glass house 13 were resistant.

Nogay and Yorganei (1984) reported that of 209 cucurbit samples with virus-like symptoms collected in 1979 and 1980, 142 contained cucumber mosaic virus, 118 water melon mosaic virus, 2 and 9, CMV+WMV-2. Physical properties and particle-dimensions of the viruses were determined.

A new structure designated CMV-K obtained from melon in Ahvaz was characterized by Elahina and Habili (1984). In the year 1985, Nagay and Yorganei investigated that cucumber mosaic virus and water-melon mosaic virus were not transmitted by seed in the cucumber, pumpkins, melon and water melon cultivars. The CMV induced local and systemic infection on cucumber, pumpkin and melon, without local lesions on inoculated leaves.

Atiri (1985) isolated a virus from fluted pumpkin (*Telfairia occidentalis*) in Nigeria, which produced symptoms in some members of *Salanaceae* and *Leguminosae* and was transmitted by *Aphis spiracola*. Their properties distinguish it from telfairia mosaic virus, which neither caused symptoms in members of their families, nor transmitted by insects. The virus in crude sap or purified preparations resulted with antiserum to CMV but not with antisera to several common viruses in Nigeria. Electron microscopy examination revealed isometric particles of 29±1 nm diameter. These properties confirmed that the virus is an isolate of CMV.

Bedlan (1985) studied the variation in cucumber mosaic virus symptoms with temperature on cucumber, melon pepper (capsicum sps.) tomato, spinach, celery and lettuce. He pointed that latent infection and weed host e.g. *Stellaria* and *Mentha* spp. play important role in the aphid transmission of this non-persistent virus.

Rosemeyer et al. (1986) isolated cucumber mosaic and four other viruses from field grown buffalo gourd (cucumbita foetidissima) near. Tuesen Az Both single and mixed viral infections were associated with symptomatic plants grown in field. Viruses were distinguished from one another by mechanical or insect transmission, particle morphology,

experimental host range and serology. Four were mechanically transmissible (CMV, water melon mosaic virus 1 squash mosaic virus 2 and water melon curly mottle virus) while the fifth, lettuce infections yellow virus is exclusively white fly transmissible. Although these plant viruses are known to infect cultivated cucurbits, an investigation of naturally occurring viruses of buffalo gourd in Arizona had not been undertaken.

Haack (1986) studied the ecology of cucumber mosaic virus. He found that samples from several thousands cultivated and wild plants in Aschersleben and Dresden, yield 609 isolates of N group of CMV. During the summer the preportion of U group of CMV increase in certain crops to > 50%. It is suggested that the relative frequencies of the virus groups may be related to their thermosensitivity. No structure appeared to be specially adapted to particular plants or weeds nor was there evidence of particular isolates among epidemics.

Garcia-arenal *et al.* (1987) analysed the nucleotide sequence of six satellite RNA's of CMV differing in their pathological properties. They concluded that primary sequence and secondary str. alternations do not correlated with differences in pathogenicity.

Gu *et al.* (1987) produced and characterized the monoclonal antibodies against CMV, belongings to the 1gM sub

group, were obtained by fusion of sp 20 myeloma cells with spleen cells from BALB/C mice immunised with CMV. They were specific to CMV and cross reacted with CMV-Q, CMV-P and CMV-B but not with CMV-6 and TAV.

Katul and Makkouk (1987) detected Zucchini yellow fleek virus, Zucchini yellow mosaic virus papaya ring spot (type w) virus, water melon mosaic II virus and cucumber mosaic virus in cucurbits from lebanon and Syria. More than one virus was found in 66.4% of the samples. Detection by ELISA, using 0.1M phosphate+0.1M EDTA buffer at pH 7.4, was the most efficient for all the viruses. Simultaneous incubation of the leaf sap and enzyme-conjugate reduced the assay period by at least 4h cross reactivity was detected between PRS (type w) V antigen WM IV and ZYMV antisera and between ZYFV antigen and ZYMV antiserum.

Hseu et al. (1987) reported that in 1985, 583 leaf samples were collected from cucurbits in Taichung, Yuchia-Nan and Kao-ping Taiwan. These include cucumber, Luffa spp. bitter gourd, pumpkin and bottle gourd (*Lagenaria leucantha*). Direct ELISA was used to detect Zucchini yellow mosaic poty virus water melon mosaic 1 poty virus and cucumber green mottle mosaic tobacco virus. ZYMV was rare in cucumber, Luff spp. B.hispida and pumpkin. In 1986, 908 samples from the same

species were tested for the above viruses plus water melon mosaic II poty virus and cucumber mosaic cucumo virus in an entended area covering Hwa-tung and penghu islands, ZYMV was most prevalent then WM IV followed by CMV, but WM IV was only predominant *L.siceraria*, *L.lacantha* WM IV was only detected in Hwa-Tung samples and no ZYMV was detected on Penghu Islands ZYMV thought to be the most important virus in cucurbit cultivation in Taiwan.

Yang et al. (1987) reported that isolates causing small necrotic lesions (SNL-isolates) and large chlorotic lesions (LCL-isolates) were obtained from cucumber leaves showing vein banding symptoms. On the basis of host range, inclusions characters, aphids transmission, serological relationships and particle morphology, the SNL isolate was identified as cucumber mosaic virus and the CL isolate as Zucchini mosaic virus. Both the viruses were transmitted by the cotton aphid in a non-persistent manner.

Yoshida and Iizuka (1987) isolated cucumber mosaic virus form cucurbiteous plants like squash, bottle gourd (*L.siceraria*) and cucumber.

Erdiller and Ertung (1988) reported the occurrence of cucumber mosaic virus on musk melon in Ankra province. Structures 5 and 6 of cucumber mosaic cucumo virus and water

melon mosaic poty virus 1 and 2 were isolated from melon in 1981-84 and identified by host range, serology, physical properties and EM.

Owen and Palukaitis (1988) while characterizing the CMV, divided RNA's into two groups, on the basis of their ability to hydridize CDNA of either Fny CMV RNA or WL-CMV RNA from its 13 strs. The extent of cross hybridization within one of these groups was analysed by an RNA protection assay. The patterns of RNA fragments protected from digestion were specific for each CMV str. and revealed the extent and location of heterogenicity among the viruses as well as within the Fny-CMV natural population.

Nitta *et al.* (1988) determined the complete sequence of CMV RNA_3 of str. Y and compared with the reported sequence of RNA_3 of structure Q.

Rizzo and Palukaitus (1988) determined and compared the nucleotide sequence of RNA_2 of the Fny-Str. (Sub-gp I) of CMV at both the nucleic acid and protein levels with the previously determined corresponding sequence of RNA2 of the Q Str. (Sub gp. II) of CMV. They found that Fny-CMV RNA II-2 consisted of 3050 nucleotides and contained a single open reading frame (ORF) of 2571 nucleotides where as Q-CMV RNA2 consisted of 3035 nucleotide level, there are 71%

sequence homology while at the protein level 73% sequence homology was noted between the two RNA's.

Maeda *et al.* 1988 produced monoclonal antibodies and discussed their use in enzyme-lined immunosorbent assay (ELISA). They reported that efficiency and sensitivity of virus detection was superior with MAb conjugate than with PAb and also noted that DAS ELISA with MAb conjugate could efficiently detect heterologous serotypes with higher ELISA values than PAb.

Saric and Stefanac (1988) reported the incidence and variation of CMV in four vegetables (Squash, *capsicum annuum, P.vulgaris* and *Lettuce*) from crotia. They found that all isolates of CMV caused similar symptoms typical on cucumber, *Datura Stromonium*, *N.glutinosa*, *N.meglosiphan* white burley tobacco and Chenopodium amaranticolar. Reaction of other test plants and serological tests showed that there were atleast 3str. Including 5 variants of CMV on the 4 vegetable host examined.

Romero *et al.* (1988) isolated double stranded RNA by using a modification of Moris and Dodd's method double standard RNA was obtained virtually free from contamination with other nucleic acids. They concluded that this method is especially useful for analysis of large no of mixed virus

infections and studies of cryptic viruses Satellite viruses and viroids. Other possible applications would be separation of mixed infections and the use of ds RNAs as probes in hydridization experiments.

Hayakawa *et al.* (1989) determined the complete nucleotide sequence (3369 nt) of RNA I of cucumber mosaic virus strain O(CMV-O). A comparative study of the 3 viruses revealed that CMV-O is more homologous to CMV (Sub gp. II) than to CMV-Q (Sub gp I) Fny.

Hsu et al. (1989) while investigating host reaction, serology and RNA pattern of CMV isolates found that out of 19 cucumber mosaic cucumovirus (CMV) isolates tested, 14 produced differential reactions, but were serologically indistinguishable in gel diffusion tests and western blot analysis. Agrose gel electrophoresis revealed that all isolates had similar MV for their RNA's 1-4. RNA 5 was found in 15 isolates NT9, 904, GLI and 1003 propagated in tobacco at the second transfer.

In the same year, Haase et al. (1989) developed two variants of direct antibody sand wish ELISA; as ELISA with PAbs and MAbs for detection of cucumber mosaic cucumo virus and a MAb-ELISA with 2 MAbs directed against different epitopes of CMV for specific detection of N-serotype. Mixed

ELISA was more sensitive than ELISA with poly clonal antisera in detecting CMV in crude sap of infecting plants. The Ist step procedure took less time and appeared to give a lower detection thresh hold.

Lee *et al.* (1990) detected plant viruses by protein A-enzyme linked immunosorbent assay. They found that the effective concentration of protein A was 0.1-3μg/ml in coating buffer solution and 0.001μg/ml in enzyme conjugate buffer protein A (PA) prevented specific reaction in different virus strains among cucumber mosaic cucumovirus strain O,C and Y. Viruses in different groups detected by this method are turnip mosaic poty virus, cucumber mosaic cucumo virus etc.

Richter (1990) used blocking ELISA as a confirmatory test in the detection of the cucumber mosaic virus and found that this technique is suitable for confirmatory identification of CMV.

Bansal *et al.* (1990) reported the occurrence of CMV on squash. Transmission test, host range physical properties and serology, the pathogen causing this major disease in North western India was identified as cucumber mosaic cucumovirus.

Shoji et al. (1991) investigated the infectivity of 18 cucumber mosaic cucumo virus isolates from cucumber plants in Japan. Severe symptoms suggested that the isolate could be

used in investigations of cross protection confirmed by attenuated virus.

Wahyani (1991) characterized strains of CMV from Australia by their host range symptomatology. They were classified as sub group I and II strains by a dot-blot molecular hydridization assay between their total viral RNA and selected cDNA. A range of serological tests was used to compare their isolates. Both molecular hydridization with total RNA and specific MAbs may be useful for separating isolates of CMV into sub group I and II.

Stoimenova (1991) reviewed the structure and function of cucumber mosaic cucumo virus (CMV) genome and Satellite RNA. cDNA. The possibility of protecting cultivated plants using vaccine strains of CMV (with or without cRNA) or transgenic plants expressing RNA or capsid protein was discussed.

Matsuo *et al.* (1991) reported the occurrence of melon caused by *Lagenaria* strain of CMV from Nagasaki perfecture.

Daniels and Cambell (1992) characterized CMV isolates of California. A total of 30 cucumber mosaic cocumovirus (CMV) isolates from California U.S.A. were characterized biologically (host reaction and thermosensitivity), serologically (protein A sandwich ELISA) and physically (viral capsid protein migration in PAGE, peptide mapping migration of viral

ds RNA in PAGE. All methods supposed the classification of CMV isolates into 2 main sub group. CMV-I and CMV-II peanut stunt cucumovirus, tomato aspermy cucumovirus and CMV were distinguished from each other by PAS-ELISA, peptides mapping and host reaction.

Paras *et al.* (1992) differentiated the biologically distinct cucumber mosaic isolates by PAGE of double, stranded RNA.

Ertung (1992) reported that out of 40 cucurbitaceae seed samples tested, cucumber mosaic cucumovirus was identified by DAS-ELISA. Although non precoated indirect ELISA gave higher absorbance values, both assays were suitable for detection of CMV in cucurbits.

Polak (1992) isolated cucumber mosaic virus (CMV) and tobamovirus from water of an irrigation ditch in the vegetables growing area north of prague. The achieved results give the Ist proof of the presence of CMV in surface water in czechoslovakia and pointed out possible danger of virus contamination by means of irrigation water.

By going through the literature it is revealed that CMV is the most extensively studied virus from different parts of the world. The different strains of the CMV have been collected from various places in an around Aligarh to ascertain whether they are the same virus (CMV), or different strains of CMV.

MATERIALS AND METHODS

1. Maintenance of virus inoculum

(i) Raising of test plants

All the plants were grown in clay pots of 4" and 6" diameter, filled with a mixture of soil, sand and compost in a ratio of 2:1:1. The soil mixture was sterilized by autoclaving for one hour at a pressure of 20 lbs per square inch. The clay pots were sterilized by rinsing in 4% formalin solution and the soil mixture autoclaved 24 hours earlier and sieved before use.

Seeds were sown in 12" clay pots of raising seedlings except for plants belonging to cucurbitaceae and leguminosae which were raised singly by direct sowing in clay pots. Seedlings were transplanted singly in clay pots of 4" and 6" diameter at 2-3 leaf stage, when they were about 2 weeks old.

For inoculation, the plants were used two weeks after transplantation except the species of *Cucurbitaceae* and *Fabaceae* families, which were inoculated at seedling stage. All the plants were kept in an insect proof glass house.

(ii) Virus culture:

Young leaves of naturally infected bottle gourd (*Lagenaria siceraria* dust showing symptoms of mosaic found growing in and adjoining areas of Aligarh were macerated in a morter with pestle adding 0.1M phosphate buffer in the ratio of

1:2. The macerate was filtrate through double layered cheese cloth and was centrifuged for 10min at 6,000 rpm. The supernatant thus obtained is a standard inoculum. This standard inoculum was used to inoculate the seedlings of *Lagenaria siceraria* in order to maintain the culture. To ensure the biological purity of virus and to eliminate the possible contaminating viruses from the culture, several dilutions were made mainly (10^{-1} to 10^{-4}) and inoculated on the seedlings of bottle gourd. The highest dilution which develops mosaic symptoms, were used further for maintaining pure culture. Periodic checks were made on assay host, *Chinopodium-amaranticolar* to ensure biological purity of the virus.

2. Transmission:

Various methods of transmission were tested to ascertain the spread of virus in nature standard methods and modifications have been carried out for the transmission studies.

(i) Mechanical

Preparation of buffers:

Different types of buffers were prepared by using the methods standardized by Gomori (1995) various additives were also used just to increase the rate of transmission of virus. Some of these are:

a) Sodium sulphite AR

b) 2-mercaptoethanol

c) Thioglycolic acid

d) Disodium ethylene-diamine tetracetic acid (EDTA)

e) Sodium diethyl dithiocarbamate (DIECA)

Three to four lower, fully expanded leaves of the plants predusted uniformaly with carborundum (500 mesh) were used for mechanical inoculation of the virus. The standard inoculum was applied gently but firmly on the upper surface of the leaves with the help of forefinger by keeping the other hand beneath the leaf to be inoculated. The inoculated leaves were rinsed with gentle stream of water before the inoculum on the surface of the leaves dried up.

(ii) Biological Transmission

Attempts were made to find out the vectors of virus in the field. Transmission by insect, soil, dodder (*Cuscuta.* spp.), seeds grafts and pollen were studied.

(a) Insect Transmission

Transmission by Aphids

Adult aphids found transmitting the disease during preliminary investigations were used to study virus vector relationship (non-persistent, semi-persistent, or persistent).

Raising of virus free Aphids

Viviparous adult were starved for 2,4,6 and 8 h at room temperature in a petridish and then placed upon a detached leaf of an appropriate healthy host in a petridish. The atmosphere inside the pateridish was made humid by covering the inner surface of the petridish with wet filter paper. Newly born nymphs were transferred to a fresh and healthy test plant. The aphid colonies thus developed were used as healthy colonies of the virus free aphids. The aphids from one plant to other were transferred with the help of moistened tip of camel's hairs brush type A, No, 1. Colonies of virus free aphids were raised on suitable host plant in cages having wooden frames. The top and two sides of the cage were chosen by glass and the remaining sides were closed by wire gauze. A fluorescent tube was fixed in the cage to keep the aphids under long day conditions to get the apterous (wingless) aphids. The plants were kept on a zinc tray and the bottom of the tray was covered with a layer moist sand to prevent the passing of the aphids through chinks between the tray and the rim of the cage.

(iii) Mode of Transmission

Non Persistent

Pre-acquisition starvation period	1 to 2 h
Acquisition access period	2 to 5 min

Inoculation access period	24h
Number of aphids/plant	10

The nymphs were starved for 1 to 2 h in a petridish having the inner surface covered with a wet piece of filter paper before an acquisition access period of 2 to 5 min on the leaf of the diseased plant. After allowing acquisition feeding time, the nymphs in batches of 10 were transferred to each healthy seedlings and the plants were covered with Leztz cages for an inoculation access period of 24h. the nymphs after the end of inoculation access were killed by spraying with 0.02 percent cypermethrine (insecticide) and the plants were kept in an insect proof glass house for the development of symptoms. Back inoculation from each plant were made to an appropriate local lesion host, i.e. *Chenopodium amaranticolor*.

Persistent

Acquisition access period	24h
Inoculation access period	48h
Number of aphids/plant	10

The virus free aphids, without subjecting them to starvation were allowed 24h acquisition feeding time on diseased leaves. After the completion of acquisition feeding, 10 aphids were transferred to each test plant where they were

given an inoculation feeding period. Aphids were killed by spraying an insecticide (cypermethrine 0.02% solution). The test plants were kept in an insect proof glasshouse to observe the development of symptoms. Back inoculations from the plants on which aphids were given inoculation feedings were made on a local lesion host i.e. *C.amaranticolor.*

(b) Transmission by whiteflies:

Whiteflies (*Bemisia tabaci* Genn.) Collected from field were caged on a healthy plant of *N.glutinosa* for egg laying. After 10 days the adults were removed from the cage. New born whitefly adults developing after 7-8 days were allowed further multiplication. Insect colonies so raised were virus free and used for transmission studies.

Handling of whiteflies:

The methods described by Rathi and None (1974) was used for handling whiteflies

Transmission:

Non-viruliferous white flies were allowed acquisition and inoculation access period of 24h each on diseased and healthy plants, respectively cypermethrine. (0.02%) was sprayed to kill the white flies after inoculation. The test plants were kept for observation of symptoms.

(c) Transmission by Dodder

Seeds of dodder (*Cuscuta reflexa* Roxb. and *C.chinensis* Lam.) were germinated on moist filter paper placed in petridish and then transferred in 4" clay pots, sterilized with form mixture. When the dodder plants were about 6" long, they were trained on a suitable host plant susceptible to the virus and the host plants (on which the dodder was trained) were inoculation after one week. When the dodder had been established on inoculated plant, a healthy test plant in another pot was placed near the pot (having inoculated plant with dodder established on it) and the tips of the branches of dodder were detached, placed in the axial of the healthy test plant and allowed to established there. The plants were left as such for about 2 months to develop the symptoms. Back inoculation was made on *C.amaranticolor* to confirm the presence of virus.

(d) Soil Transmission

Soil was collected from around the root zone of virus infected bottle gourd plants and was divided into two parts. One part was autoclaved at 20lb for an hour which served as control and other part left as such. The seeds of bottle gourd were sown in both sterilized and unsterilzied soils contained separately in 6" clay pots. Symptoms were observed till a period of 2 months after sowing. Infectivity tests were also

carried out on bottle gourd (*L.siceraria*) to ascertain the presence of virus in them.

(e) Seed Transmission

Seeds collected from virus infected and healthy plants of bottle gourd were sown separately in clay pots containing sterilized soil and were kept in insect proof glass house. Plants were observed for 2 months after sowing. Infectivity test were also carried out on bottle gourd to ascertain the presence of virus in them.

3. Selection of local lesion host

The sap prepared from the young infected leaves of bottle gourd was inoculated on various local lesion hosts viz. *Chenopodium amaranticolor, C.quinoa, C.album, C.murale Datura metel, D.stramonium* L., *Gomphrena Glubooa* L., *Nicotiana glutinosa.*

The local lesions developed (if any) on the inoculated leaves of each lesion host were counted and compared to select the most suitable one.

4. Host Range and symptomatology

Plants belonging to different families were screened for the susceptibility to the virus by mechanical inoculation using standard inoculum. At 4-6 leaf stages, atleast 3 plants of each species/ cultivar were inoculated with standard inoculum and

the same number of plants were left as control. Inoculated plants were observed till 40-50 days for the development of the symptoms. All inoculated plants, including those which did not show sign of the disease were tested by back inoculation on *C.amaranticalor* to find out latent infection, if any.

5. Properties of the virus in plant sap:

Biophysical properties:

The following technique for bio-physical properties (described by Noordam, 1973) were employed.

(a) Dilution End Point (DEP):

The sap of virus infected bottle-gourd plants was obtained by the method described earlier and tenfold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) made of the sap by the addition of double distilled water. Three plants of *C.amaranticolor* having 8 leaves of equal size and same leaf area were inoculated by the each dilution. After 4 days of inoculation local lesion development on the leaves were counted.

(b) Thermal Inactivation Point (TIP):

The sap was obtained from young infected leaves of bottle-gourd by the method mentioned earlier. Two ml of sap in each of the 11 test tubes was taken one test tube was left as such at room temperature and other were heated at 40^0, 45^0, 50^0, 55^0.....85^0 and 90^0 for 10 min in a water bath

and cooled immediately in running water. the test tubes were held in water bath in such a way that the water level in the bath remained 2-3cm above the sap level. Three plants each of *C.amaranticolor* having 8 leaves of equal size were inoculated with the sap of each test tube and the local lesions were counted after 4 days of inoculation.

(c) Longivity in vitro (liv):

Longivity in vitro is defined as the time that virus in crude juice kept at room temperature remains infective. Sap was prepared by macerating the young infected leaves of bottle-gourd and was divided into two parts. One of them was stored at room temperature (20 ± 5^0C) while the other at 4^0C. Three plants of *C.amaranticolor* having 8 leaves of equal size were inoculated with the sap separately at 6h intervals upto several days. Local lesions developed on inoculated leaves were counted.

RESULTS

1. Natural Symptoms:

Naturally infected plants of bottle gourd, *L.siceraria* showed characteristic mosaic (irregular light and dark green mottling) on limited portion or on entire leaf surface. At advance stage of infection, reduction in flower and fruit production and general stunting of growth were observed.

2. Transmission:

(a) By Sap:

The virus causing cucumber mosaic disease was readily transmitted by sap extracted in 0.1M phosphate buffer pH 7.0 from bottle gourd to different species of cucurbitaceae and some other families. There was 100% transmission of the disease by sap inoculation using carborandum (500 mesh) as an abrasive.

(b) By aphids:

Three aphid species viz. *Myzus persicae* sulz., *Aphis gossypii* Glov., and *Aphis craccivora* Koch. were used for examining the transmission of virus. Nymphs from the healthy colonies were starved for 2-3 h and were given an acquisition

access to diseased leaves of *L.siceraria* from 1 to 2 minutes to 24 h. There after, these aphids were placed on young healthy seedlings of bottle gourd from 1 min to 24 h (inoculation feeding period). Eight to ten aphids were used per test plant. The acquisition and inoculation feeding were performed in small cages. And after the inoculation feedings the aphids were killed by spraying 0.2% dimecron. The inoculated plants were then transferred to glass house for observing the development of symptoms. Back inoculation from each inoculated plant to *L.siceraria* were made to confirm the presence of virus, if any.

The results of the expt suggests that virus is transmitted by the *Aphids Aphis craccivora*, *A.gossypii* and *M.persicae* in a non-persistant manners but among these *M.persicae* is the most efficient vector.

(c) By white fly

White fly, *Bemisia tabaci* Genn., failed to transmit CMV strains even after increasing the no. of white flies per plant upto 15. Neither *L.siceraria* plants showed any symptom even after 30 days of inoculation nor they gave positive results on back inoculation to *C.amaranticolor* plant to show the presence of virus.

(d) By dodder:

The 2-species of dodder, *Cuscuta reflexa* Roxb. and *C.chinensis* Lam. found commonly in and around Aligarh district were tried to transmit the cucumber mosaic virus from infected to healthy plants. Symptoms of virus were recorded on healthy and young plants connected with diseased ones, by each of the dodder species even after 2-months. Back inoculation from each plant to *L.siceraria* was made to confirm the presence of the virus but negative results were achieved in all the cases which shows that the virus causing mosaic namely CMV is not transmitted by dodder.

(e) By Soil:

About 10 seeds were sown in 6 inche clay pots containing soil collected from around the roots of virus infected bottle gourd plants. The development of symptoms were observed upto 2-months. Soil from around the healthy bottle gourd was treated as control. No. transmission of virus through soil was noticed as the tested plants neither showed any visible symptoms nor evoked any symptoms on back inoculation from the sap of infected soil plants to healthy cotyledons of bottle gourd Table 2.1

Plant	Soil source	No of seeds sown	No of seeds germinated	No. of plants infected	%age of transmission
Bottle gourd (*L.Siceraria*)	CMV infected plants	10	8	Nil	0
	Healthy plants	10	9	Nil	0

Result based on 3-experiments.

(f) By seed:

About 10 seeds of bottle gourd were tested for possible transmission of virus by seeds. The results of experiment revealed that CMV get transmitted through seed but in variable extents.

Plant/cultivar /source	Total No. of seeds grown	No. of seeds germinated	No of infected plants	%age of infection
L.siceraria	20	18	14	77.77
L.siceraria.	10	10	4	40

Result based on three trails.

3. Host Range and Symptomatology:

The experimental host range of CMV was investigated by mechanical sap inoculation from infected plants to various species and cultivars of plants belonging to different families.

The sap was prepared in 0.1M phosphate buffer pH 7.0 from infected leaves of bottle gourd. Back inoculations from all inoculated plants were made on *Chenophodium amaranticolor* to ascertain the presence of virus. Following plants developed symptoms on inoculation and virus was recovered on back inoculation to test plant.

Amaranthaceae

Amaranthus spinosus

A.tricolor

A. caudatus L.

Gomphrena globosa

Asteraceae

Calendula officinalis L. cv. Suttons Lemon

Chrysanthemum indicum L.

Helianthus annuus L.

Sonchus oleraceus L.

Brassicaceae

Brassica compestris L.

Brassica rapa L.

Brassicea oleracea cv. Pusa snowball

Raphanus sativus L. cv. Pusa Rashmi

Chenopodiaceae

Chenopodium amaranticolor coste and Reyn.

C.murale L.

C.quinoa L.

C. album L.

Spinacea oleraceae L.

cv. palang sag

cv. pusa jyoti and palang sag.

Cucurbitaceae

Citrullus vulgaris L.

Cucumis melo

Cucumis sativus L.

cv. pointsetee SIG

cv. poona Kheera

Cucurbita maxima L.

Cucurbita moschata L.

Cucurbita pepo L.

Luffa cylindrica L. Roem

Momordica charantia L.

Malvaceae

Abelmoschus esculentus L. Moench cv. Pusa Sawani

Papilionaceae

Pisum sativum L.

Trigonella foenum-graecum L.

Viccia faba L.

Viccia sativa L.

Vigna radiata L.

Vigna sinensis L.

Solanaceae

Capsicum annuum L.

var. Ruby King, Chinese giant

Capsicum frutescens.

Nicotiana tabacum L.

cv-Harrison special

cv-Jayshree

cv-samsun NN.

cv- Bopali pakra

cv-Bidi Anand-2

Solanum melongena L.

cv-pusa purple long

cv-Black beauty

Datura metal L.

Datura stramonium L.

Lycopersicum lycopersicon L. Karst.

cv-Pusa Ruby

cv-Punjab Kesari

N. megalosiphon Heurek et Muell

N.debeyi

N.glutinosa L.

N.rustica schrank

Nicotiana clevelandii

Physalis Floridana

Physalis peruviona

Solanum nigrum L.

Solanum tuberosum

Apiaceae

Apium graveolens L.

Coriandrum sativum L.

Daucus carota L.

3.1 Right, a naturally healthy leaf of *L.siceraria*
Left, a naturally infected leaf of *L.siceraria*
Showing mosaic, mottling and raised green area

3.2 Infected leaves of *L.siceraria* showing mosaic, vein clearing and raised green areas.

3.3 Infection plant of *Dahlia* showing puckering and deformation of leaves.

3.4 Infected plant of *L.siceraria* showing chlorosis and mosaic.

3.5 *Nicotiana debeyi*; systemic symptoms appeared in the form of yellow net mosaic, reduced lamina and deformed leaf.

3.6 *L.siceraria*, inoculated leaves showed dark green mosaic.

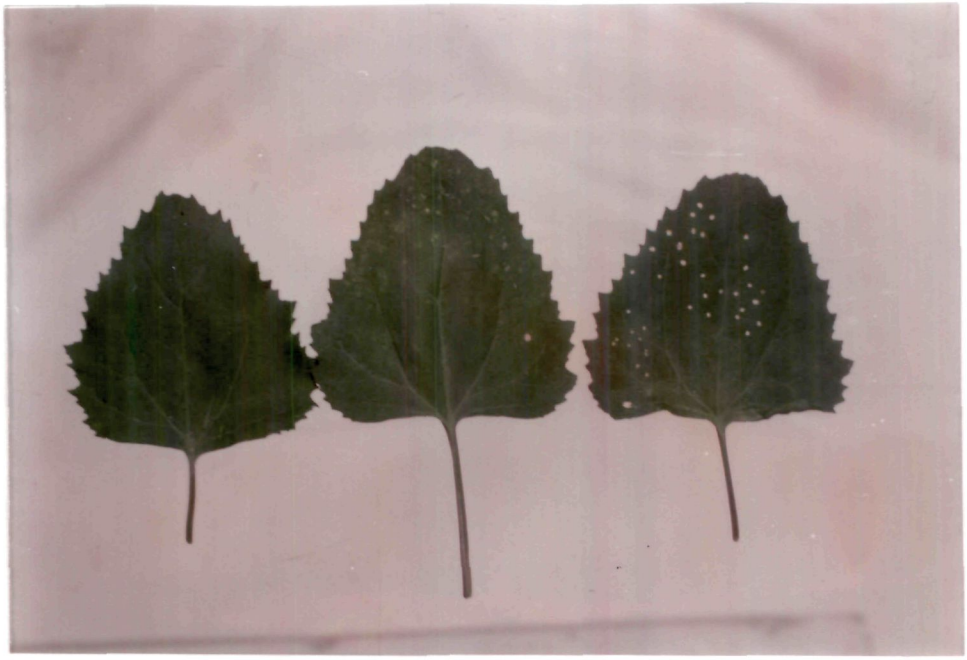
3.7 Infected plant of *L.siceraria* showing severe infection in the form of stunting, retarted growth, chlorosis and deformation of leaves. At later stage leaves showed shoe-string formation.

3.8 Infected plant of *Petunia* showing mosaic and overall stunting.

3.9 Infected plant of *Spinacea oleracea* cv. Palang sag showing upward curling of leaves and mosaic.

3.10 *C.amaranticolor* showing diffused chlorotic local lesions.

3.11 Infected plant of *Capsicum annuum* showing puckering and deformation of leaves.



3.12 *C.amaranticolor* leaves showing discrete local lesions.

3.13 *L.siceraria*- inoculated leaf showing chlorosis and vein necrosis.

3.14 Right-a healthy leaf of *Lycopersicon lycopersicum* left-inoculated leaves of *Lycopersicon lycopersicum* showing necrotic local lesions.

3.15 *Cucurbita moschata*- an inoculated plant showing marginal mosaic on young leaves of plants.

3.16 Inoculated plant of *Luffa cylindrica* showing discrete local lesions.

3.17 *Cucumis sativus* leaves showing systemic infection in the form of deformation on new emerging leaf.

3.18 Fruit from inoculated plant of CMV showing highly deformed fruit, also reduced in size.

4. Selection of local lesion host:

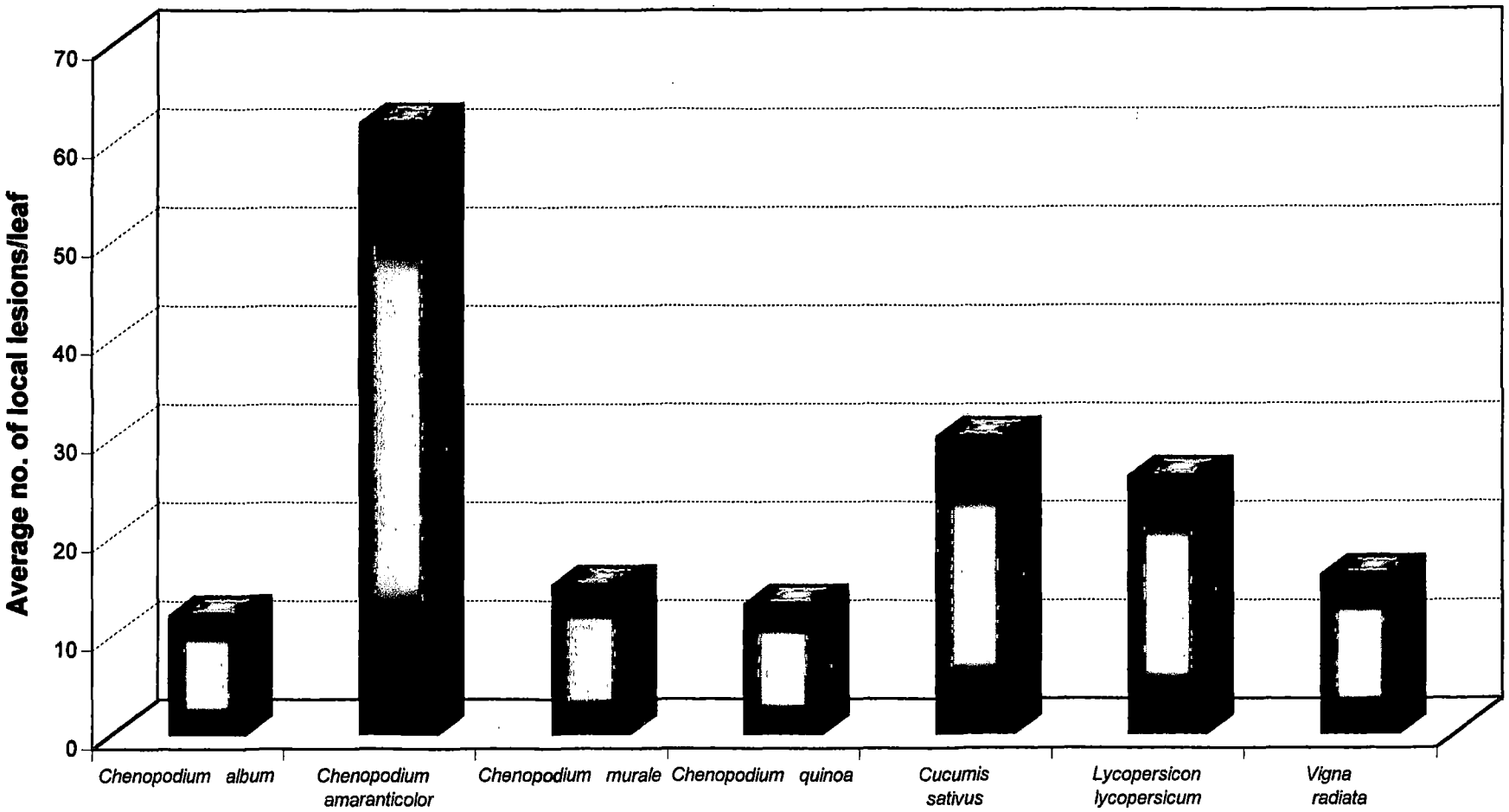
Several local lesion hosts viz. *Chenopodium-album*, *C.amaranticolor*, *C.murale*, *C.quinoa*, *Cucumis sativus*, *Vigna unguiculata* and *Lycopersicon lycopersicum* were used and then compared for the selection of the most suitable one. The inoculum was prepared from bottle gourd and local lesions were counted 4-5 days after inoculation.

Table 4.1 Number of local lesion hosts of CMV.

Local lesion hosts	Average no. of local lesions/leaf*
Chenopodium album	12
C.amaraticolor	62
C.murale.	15
C.quinoa	13
Cucumis sativus	30
Lycopersicon lycopersicum	26
Vigna unguiculata	16

*** Average no of local lesions/leaf based on 3 plants with each having 4 fully expanded leaves.**

Fig. 4.1: Average number of local lesions on different local lesion hosts of CMV.



On the basis of the data, it is apparent that *C.amaranticolor* gave the best results as the local lesions produced were higher than the others. Hence *C.amaranticolor* was used as a local lesion host in all the studies.

Here in the studies I have studied the general host reported already in the literature. However, the hosts which were found susceptible to these Aligarh isolates were also taken into the consideration for the strains of CMV.

5. ***Datura stramonium***

Strain A shows different local lesions which is generally in the form of concentric rings, small spots, necrotic lesions etc. Systemic symptoms appeared in the form of vein clearing and mottling with necrosis and distortion. Other two strains show only systemic symptoms which appear in the form of clearing of veins followed by general mosaic.

Solanum tuberosum

Infection could not be obtained on potato plants with any one of the strains.

Lycopersicum esculentum **var. Pusa Ruby:**

Strain A cause local lesion in the form of necrotic lesions and systemic symptoms is in the form of curling and distorted leaves. No local lesion have been found in other strains. No doubt the other strains show systemic lesion. All strains produced fern leaf.

Phytolacca amaricana

Local lesions in all the four strains appear in the form of diffused chlorotic symptoms. Same is the case with systemic symptoms which appear in the form of general mosaic.

Beta vulgaris

Sugar beet var. katari-73 shows some symptoms with all the three strains. However the leaves inoculated with strain A died. Local lesions appear in the form of necrotic or chlorotic which soon becomes systemic consisting of mosaic with dwarfing of growth and reduced leaf size.

Cucumis sativus **L.c.v. poona kheera**

Strain A differed from the others in causing circular, chlorotic local lesions in cucumber. Systemic symptoms appeared in the young leaves and later changed into a general

mosaic. Leaves are very much infected, reduced in size and show distortion. The other strains namely strain B and strain C produced systemic symptoms which are less severe than those caused by the strain A.

***Citrullus vulgaris* var. fistulosur cv. Dilpasand**

Strain A cause water soaked local lesions which later become necrotic and chlorotic. No such symptoms have been observed in other types of strains.

Nicotiana glutinosa

Strain A shows local lesions mainly necrotic. Leaves are mottled and show distortion. The other strains produce systemic symptoms, mottling and leaf distortion.

***Nicotiana tabacum* variety Bopali pakra**

Strain A shows necrotic local lesions. The first systemic symptom is clearing of veins followed by distortion and curling of leaves.

***Lathyrus odoratus*:** None of them infect sweet pea

Pisum sativum: All the strains cause local as well as systemic symptoms in peas. Strains cause necrotic lesions and general yellowing, followed by severe chlorosis.

Phaseolus vulgaris

Local lesions may appear in the form of spots and rings. No systemic symptoms occur.

6. Properties of the virus in plant sap

Johnson (1927) suggested about thermal inactivation point, dilution end point, longevity in vitro in identification of plant viruses. These gave an idea about the stability concentration and other properties of the virus in plant sap and provided information about the best environment to keep the virus and maintain infectivity. Although these studies have restricted values (Ross, 1964). They are of utmost importance in determining the procedure for the purification and characterization of a virus.

Studies on these properties were carried out using *Lagenaria siceraria* as donor of the virus and tests were made on local lesion host *C. amaranticolor*.

(a) Dilution end point (DEP) of strain A

The virus in crude sap was found to be infectious at a dilution of 10^{-4} but no local lesions were observed when the sap was diluted to 10^{-5} (table No.). Therefore the dilution end point of the virus is between 10^{-4} to 10^{-5} Table 6.1.

Table 6.1: Dilution end point (DEP) of strain A

Dilution	Number of plants inoculated	No of plants infected	Local lesion number/leaf*
Undiluted	5	5	204
10^{-1}	5	4	110
10^{-2}	5	4	52
10^{-3}	5	3	22
10^{-4}	5	2	08
10^{-5}	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

(b) Thermal inactivation point (TEP) of strain A

In crude sap, the virus was still infective after being heated for 10 min at a temperature of 70^0C but lost its infectivity at 75^0C Table 6.2.

Table 6.2: Thermal Inactivation point (TEP) of strain A

Temperature (^{0}C)	No of plants inoculated	No of plants infected	Local lesion number/leaf*
Unheated	5	5	198
40	5	5	182
45	5	5	154
50	5	4	129
55	5	4	119
60	5	3	114
65	5	3	84
70	5	2	62
75	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

(c) Longevity in vitro (LIV) of strain A

Two samples of crude sap (30ml each), obtained from infected leaves, were kept in two sterilized vials. One was kept at room temperature (20±5) while other in a refrigerator (4^{0}C). Each samples was assayed on *Lagenaria siceraria* after as specific period of respective storage Table 6.3.

Table 6.3 (a): Longevity in vitro (LIV) of strain A

At 4^0C temp. storage in hours	No of plants inoculated	No. of plants infected	No. of local lesions/leaf*
00	5	5	199
06	5	5	197
12	5	5	180
18	5	5	172
24	5	4	159
30	5	4	152
36	5	4	147
42	5	4	141
48	5	4	128
54	5	4	127
60	5	3	114
66	5	3	111
72	5	3	107
78	5	3	102
84	5	3	100
90	5	3	92
96	5	2	84
102	5	2	71
108	5	2	69
114	5	2	64
120	5	2	59
126	5	2	57
132	5	2	50
138	5	2	46
144	5	2	35
150	5	2	27
156	5	0	0

*Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.

Table 6.3 (b): Longevity in vitro (LIV) of strain A.

At room temp storage in hours	No of plants inoculated	No. of plants infected	No. of local lesion/leaf*
00	5	5	199
06	5	5	180
12	5	5	177
18	5	5	161
24	5	5	150
30	5	5	143
36	5	4	120
42	5	4	101
48	5	4	91
54	5	4	76
60	5	4	64
66	5	4	59
72	5	4	40
78	5	3	37
84	5	3	20
90	5	3	17
96	5	2	14
102	5	2	6
108	5	2	4
114	5	2	2
120	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

Dilution end point (DEP) of strain B

The virus in crude sap was found to be infectious at a dilution of 10^{-4} but no local lesions were observed when the sap was diluted to 10^{-5} (table No.). There for the dilution end point of the virus is between 10^{-4} to 10^{-5} Table 6.4.

Table 6.4: Dilution end point (DEP) of strain B.

Dilution	No. of plants inoculated	No. of plants effected	No. of local lesions /leaf*
Undiluted	5	5	184
10^{-1}	5	5	170
10^{-2}	5	4	83
10^{-3}	5	3	32
10^{-4}	5	1	2
10^{-5}	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

Thermal inactivation point (TEP) of strain B

In crude sap, the virus was still infective after being heated for 10 min at a temperature of 60^0C but lost its infectivity at 65^0C Table 6.5.

Table 6.5: Thermal activation point (TEP) of strain B

Temperature (^{0}C)	No. of plants inoculated	No. of plants effected	No. of local lesions /leaf*
Unheated	5	5	179
40	5	5	142
45	5	3	129
50	5	3	119
55	5	2	99
60	5	2	67
65	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

Longevity in vitro (LIV) of strain B

Two samples of crude sap (30ml each), obtained from infected leaves, were kept in two sterilized vials. One was kept at room temperature (20±5) while other in a refrigerator (4^0C). Each samples was assayed on *Lagenaria siceraria* after a specific period of respective storage Table 6.6. (a)

Table 6.6 (a): Longevity in vitro (LIV) of strain B

At 4^0C temp storage in hours	No of plants inoculated	No. of plants infected	No. of local lesions/leaf*
00	5	5	176
06	5	5	170
12	5	5	160
18	5	5	159
24	5	5	154
30	5	4	149
36	5	4	146
42	5	4	140
48	5	4	138
54	5	4	134
60	5	4	129
66	5	3	120
72	5	3	118
78	5	3	110
84	5	3	104
90	5	3	99
96	5	3	88
102	5	3	71
108	5	3	60
114	5	2	44
120	5	2	36
126	5	2	31
132	5	2	28
138	5	2	21
144	5	1	15
150	5	0	0

*Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.

Table 6.6 (b): Longevity in vitro (LIV) of strain B.

At Room temp storage in hours	No of plants inoculated	No. of plants infected	No. of local lesions /leaf*
00	5	5	168
06	5	5	164
12	5	5	158
18	5	5	156
24	5	5	144
30	5	4	142
36	5	4	139
42	5	4	136
48	5	4	131
54	5	3	122
60	5	3	102
66	5	3	98
72	5	3	91
78	5	2	72
84	5	2	65
90	5	2	63
96	5	2	61
102	5	2	46
108	5	2	30
114	5	1	10
120	5	0	0

*Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.

Dilution end point (DEP) of strain C

The virus in crude sap was found to be infectious at a dilution of 10^{-3} but no local lesions were observed when the sap was diluted to 10^{-4} (Table 6.7). Therefore the dilution end point of the virus is between 10^{-3} to 10^{-4} Table 6.7.

Table 6.7: Dilution end point (DEP) of strain C.

Dilution	No. of plants inoculated	No. of plants infected	No. of local lesions /leaf*
Undiluted	5	5	82
10^{-1}	5	4	71
$.10^{-2}$	5	3	30
10^{-3}	5	1	09
10^{-4}	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

Thermal inactivation point (TEP) of strain C

In crude sap, the virus was still infective after being heated for 10 min at a temperature of 50^0C but lost its infectivity at 55^0C Table 6.8.

Table 6.8: Thermal activation point (TEP) of strain C.

Temperature (^{0}C)	No. of plants inoculated	No. of plants infected	No. of local lesions /leaf*
Unheated	5	5	79
40	5	5	70
45	5	5	41
50	5	4	17
55	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

Longevity in vitro (LIV) of strain C

Two samples of crude sap (30ml each), obtained from infected leaves, were kept in two sterilized vials. One was kept at room temperature (20±5) while other in a refrigerator (4^{0}C). Each samples was assayed on *Lagenaria siceraria* after as specific period of respective storage Table 6.9.

Table 6.9 (a): Longevity in vitro (LIV) of strains C.

At 4^0C temp storage in hours	No of plants inoculated	No. of plants infected	No. of local lesion/leaf*
00	5	5	81
06	5	5	80
12	5	5	80
18	5	5	78
24	5	5	74
30	5	5	71
36	5	5	69
42	5	4	65
48	5	4	64
54	5	4	62
60	5	4	58
66	5	4	56
72	5	4	55
78	5	3	48
84	5	3	44
90	5	3	39
96	5	3	37
102	5	3	30
108	5	3	28
114	5	3	21
120	5	2	19
126	5	2	14
132	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

Table 6.9 (b): Longevity in vitro (LIV) of strain C.

At Room temp storage in hours	No of plants inoculated	No. of plants infected	No. of local lesion/leaf*
00	5	5	81
06	5	5	79
12	5	5	78
18	5	5	71
24	5	5	62
30	5	5	59
36	5	5	52
42	5	5	40
48	5	4	33
54	5	4	28
60	5	4	27
66	5	3	19
72	5	3	17
78	5	3	13
84	5	2	09
90	5	2	08
96	5	2	05
102	5	2	02
108	5	0	0
114	5	0	0

***Average number of local lesions /leaf based on 5 plants of *C.amaranticolor* having 6 leaves each.**

DISCUSSION

No doubt, the damage of every virus is huge but the loss caused by CMV is very very high. It not only infects vegetables but also ornamentals and weeds. It's effect has been found very much prevalent in cucurbitaceae and solanaceae families. The loss of productivity and severity of infection also varies with the type of CMV strain. I have worked here in this dissertation to differentiate three strains of CMV on the basis of host range, symptomology, Transmissibility by aphids and biophysical properties. Here it has been seen that susceptibility of host varies with the type of various CMV strains.

It is transmitted mechanically by sap inoculation and also by aphids. Transmission is high, in case of aphid, *Myzus persicae*. No transmission of virus occur by soil or by dodder/*cucuta chinensis* Lam. *Cuscuta reflexa* Roxb., soil and seed.

Experimental host range studies revealed that virus under study has a moderate host range. It infects 191 species of 40 families. *Lagenaria siceraria* was found suitable as a propagation host. Several *Chenopodium* spp. reacted with localised lesions to this virus. Among them *C.amaranti*color

was found to be most suitable and was selected for quantitative assay as it gave maximum and clear local lesions.

Virus of strain A in crude sap lost its infectivity after heating at 75^0C for 10 min. but remain infective at 70^0C and lost infectivity at a dilution of 10^{-5}. It remained infective for150 hrs when stored at 4^0C and 114 hrs when stored at room temp (20 ± 5^0C).

Virus of strain B in crude sap lost its infectivity after heating at 65^0C for 10 min but remain infective at 60^0C and lost infectivity at a dilution of 10^{-5}. It remained infective for 144 hrs at 4^0C and 114hrs when stored at room temp (20 ± 5^0C).

Virus of strain C in crude sap lost its infectivity after heating at 55^0C for 10 min but remain infective at 50^0C and lost infectivity at a dilution 10^{-4}. It remained infective for 126 hrs at 4^0C and 102 hrs when stored at room temperature (20 ± 5^0C).

Though all the three strains studied showed variation in host range and biophysical properties in some way or the other but still it can not be claimed that these three strains are dissimilar without the detailed studies of serology, electron microscopy and polymerase chain reactions etc. which will be carried out in my Ph.D. work in future.

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